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Use of Bifidobacterium for Treatment and Protective from Entamoeba Histolytica Infection in Mice

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ABSTRACT

This study describes the *in vivo* effect of *Bifidobacteria* in *Entamoeba histolytica* infected BALB/c mice. Experimentally, It was noticed that daily administration live *Bifidobacteria* cells in 7 days prior to challenge with *Entamoeba histolytica* was efficiently reduce severity of infection in mice, and resolution infection was occurred in day 8th post inoculation ,with efficacy treated (75.63%) ,also excretion of *histolytica* cysts were significantly reduced in probiotic-treated groups ,and infection completely end in day 10 post-inoculation ,with efficacy treated(67.86%).

It was very clear that *histolytica* cysts decreased in metronidazole treated group with efficacy(67.56%), but infection remained until 9th day post inoculation, compared with controlled mice remained excretion of *histolytica* cysts through all experimental period. Histolopathological study showed that probiotic administration also induce intestinal lymph node proliferation.

The data demonstrates the anti-histolytical effect of probiotic *in vivo* by inhibiting the colonization of *histolytica* trophozoites and there reducing the severity of *histolytica* infection.

Keyword :*Entamoeba histolytica, amoebiasis Bifidobacteria* ,Probiotic, *Lactobacillus.*

1. INTRODUCTION

Amoebiasis is a common world wide disease in developing countries ,caused by infection with protozoan parasite *Entamoeba histolytica*[1].

According to the best estimates approximately 48 million individuals suffer from amoebiasis throughout the world[2]. In 1984 at least 40,000 people are estimated to die each year from amebic colitis and amebic liver abscess(ALA).[3]in their natural environment, trophozoites of *E. histolytica* live in the colonic region of the human intestine togather with resident microbial flora, which under normal conditions is composed of acomplex mixture of mostly anaerobic or micro aerophilic bacteria[4].

The predominant an aerobic species in this flora belong to *Bifidobacterium ,clostridium* and *Eubacterium*, whereas facultative anaerobes, such as *Echererichia coli*, *Enterobacter*and *Lactobacillus*, are among the subdominant genera[5].

It has been suggested that the bacterial flora provides anaerobic conditions or low redox potential beneficial for amoebic growth [6]. For the control of amoebiasis and its clinical manifestations,metronidazole is the most common drug used [7]. but these drug present negative secondary effects .Moreover new reports shows evidence of resistance of *E. histolytica* for this drug, on the other hand, recent researches report the use of probiotic in the treatment of infectious disease as: Giardiasis, Listeriasis, and rota virus have been considered as an option to be used in clinical medicine ,beyond nutritional option[8].

Probiotics are viable non-pathogenic microorganisms that when ingested have beneficial effects in the prevention and treatment of pathological conditions[9,10].

General mechanisms of action that have been prescribed to probiotics include competition for receptor sites on the intestinal surface, immune system stimulation ,excretion of anti microbial substance, and competition with pathogens for intra-luminal nutrients [11].

Bifidobacteria is a probiotic naturally occurring in humans, is inhabiting the gastrointestinal tract and vagina and produce beneficial acetic acids and acetic acid [12], *Lactobacilli* and *Bifidobacteria* are lactic acid bacteria (LAB) commonly found in fermented dairy products such as yogurt which exhibit probiotic properties [13].Many of these probiotic are lactic acid bacteria, and anaerobic bifidobacteria have been reported to be useful in the treatment of disturbed in intestinal microflora and diarrheal disease [14],ceding probiotic bifidobacteria to experimental animals has been reported to prevent gram- negative bacterial infections [15, 16, 17].

Some probiotic bifidobacterial strains have been reported to lessen the severity of oral shiga toxin *Escherichia coli* (STEC).infection in murine experimental reduced viability of *Cryptosporidium parvum* oocyst [20]. Most of these reports, however, utilized *bifidobacteria* to protective and treated diarrhea and disease caused by other causes like bacteria, virus or antibiotic but not by parasites[21].

Therefore my search aim to use *bifidobacteria* to prevention and therapy to diarrhea caused by *E. histolytica* in mice.

2. MATERIAL AND METHODS

2.1 Feces Samples

The present study included(50) stool samples were collected from patients infected with amoebiasis, in Arab children hospital and from some an official analysis laboratories in Baghdad city, directly a wet slide prepared by using logols iodine stain to search to *E. histolytica*[22]. The positive samples saved in cool containers, transferred to Al-Mustansiryiah University.

2.2 Parasite Purification

Method of Bingham and Meyer 1979 [23] . Has been used to purficate the parasite (cyst), cysts were suspended in phosphate buffer saline (PBS- 7.2) to a final concentration of 1×10^6 cysts/ 0.1 ml.

2.3 Bacterial Cells Preparation

Bifidobacterium sp. Was obtained from department of Biology in Al-Mustansiryiah University, organisms were grown an aerobically in De Mann Rogosa Sharpe (MRS) at 37°C for 18 hr, then the culture was centrifuged at 6000 rpm for 15min, supernatant was removed and bacterial cells were taken, washed and suspended in to cotain1×10^9 lactobacilli /ml, 0.1ml fed via orogastric gavages [24].

2.4 Animals

BALB/c mice aged 5 – 6 weeks old (18-20 gm) obtained from Animal House, medicine college- Baghdad University, were housed under standard conditions of light and dark cycle . Animals were also screened for any protozoal infection via stool examination for three consecutive days. Mice free from parasitic infections were used.

2.5 Experimental Design

Animals were divided mainly into (4) groups, each containing (6)mice

-GroupI(Histolytica-*bifidobacteria* protective): Were fed orally with a single dose of respective *bifidobacteria* (0.1 ml) contain 1×10^9 cfu for 7 days, on the 8th day a single challenge dose of $1x10^6$ *histolytica* cysts.

-GroupII(Histolytica-*bifidobacteria* treated): These animals were challenged orally with a single dose of $1x10^6$*histolytica* cysts. A 3 day after *histolytica* challenge, mice were fed orally with a single dose of *bifidobacteria* $1x10^9$ cfu/ 0.1ml.

-GroupIII(metronidazole):Animals belonging to this group were challenged orally with a single dose of $1x10^6$ *histolytica* cysts. A 3 day after *histolytica* challenge, a single dose of metronidazole o.1ml(15mg\kg)was dministered .

- Group IV(control): These mice also challenged orally with a single dose of $1x10^6$ *histolytica* cysts. A3 day after *histolytica* challenge ,then fed with a single dose of PBS -7.2 via orogastric gavages along the period of experiment .

2.6.Enumeration of *histolytica* cysts in faces

Cysts in the fecal samples of mice were enumerated as per Shukla *et al.* 2008 (25). Briefly, one gram of freshly passed fecal sample was dissolved in 10 ml of normal saline, homogenized using pestle and mortar. Cysts stained with iodine were counted on every third day using hemocytometer and were expressed as cysts.

2.7 Sufficient Treatment Calculation

Sufficient treatmentfor bacterial *Bifidobacterium* and metronidazole was measured according to method of Xiao *et al.*1996 [26].

2.8 Histopathological Study

Mice in group I and II were sacrificed by cervical dislocation and large intestine was removed aseptically, fixed in 10% buffered formalin, processed, stained with haematoxylin and eosin and were examined under the light microscope.

2.9 Statistical Analysis

The inter group variation was assessed by one way analysis of variance (ANOVA). Statistical significance of the result was calculated.

3. RESULTS

The present study was designed to assess the effect of *Bifidobacterium* as probiotic supplementation in modulating the *histolytica* cycle in BALB/C mice. It was found that orally administered *histolytica* cyst in mice could transiently colonize the gut, and infection was occur between 2-3 days among all the infected mice the infected mice beginning cyst excretion on day 3th post inoculation also none of the mice from any these groups showed any clinical symptoms like diarrhea, weight loss and death. It was found that oral administration of *Bifidobacterium* 7 days before *histolytica* inoculation (GI) significantly ($p< 0.05$) reduced the cyst excretion in mice since first day(9.4×10^2) and became *histolytica* free by day 8th post inoculation tab (1), with treatment efficacy(75.63 %) tab(2).

While *histolytica* infected mice (GII) after *histolytica* infection and oral feeding with *Bifidobacterium* significantly ($p< 0.05$) de voided cyst gradually from day 1 th 1×10^2 .The cyst count started decreasing and became *histolytica* free by day 9th PI tab(1) with treatment efficacy (67.86 %) tab (2), while compared with GIII(*histolytica* infected mice and treated with meteronidazole 15mg/ kg), the cyst count also started decreasing from day1th 11×10^2 but became *histolytica* free on day10th PI tab (1) with treatment efficacy(67.56%) .

The GIV (control group) the infected mice maintain cyst excretion and no difference noticed in number of cyst excretion till day12 th post inoculation PI.

The histopatholgical study shows that the lymph node in the intestinal tissue became obviously larger in mice inoculated with *bifidobacterium*. Fig(1).

Table 1: Mean of *E.histolytica* cysts in treatment and control groups ±SD ×10^2

Groups	Day after Treatment											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
G1*	9.4±0.70	7.8±0.83	6.0±0.70	4.6±0.54	2.4±0.54	1.4±0.54	0.4±0.57	0	0	0		
G2*	10±0.83	11.8±1.78	6.6±0.89	5.o±0.70	3.4±0.89	2.4±0.54	1.6±0.54	1±0.54	0.4±0.54	0		
G3*	11.0±1.0	9.0±0.70	7.2±0.83	5.6±0.54	4.0±0.70	3.0±0.70	1.6±0.54	1.4±0.54	0.6±0.54	0		
G4*	11.2±0.83	13.4±0.89	12.6±1.51	14.6±1.51	16.0±0.70	17.0±0.70	15.2±0.83	10.0±1.14	11.2±0.83	10.2±0.83	11.0±1.0	11.8±1.78

*significant($p<0.05$)

Table 2 :Sufficient Treatment for Treatment Groups

Type of treatment	Used dose	treatment of efficiency%
Bifidobacteria treatment	1×10^8 cell/ml	67.86
Bifidobacteria protective	1×10^8 cell/ml	75.63
metronidazole	15mg/kg(0.1ml)	67.56

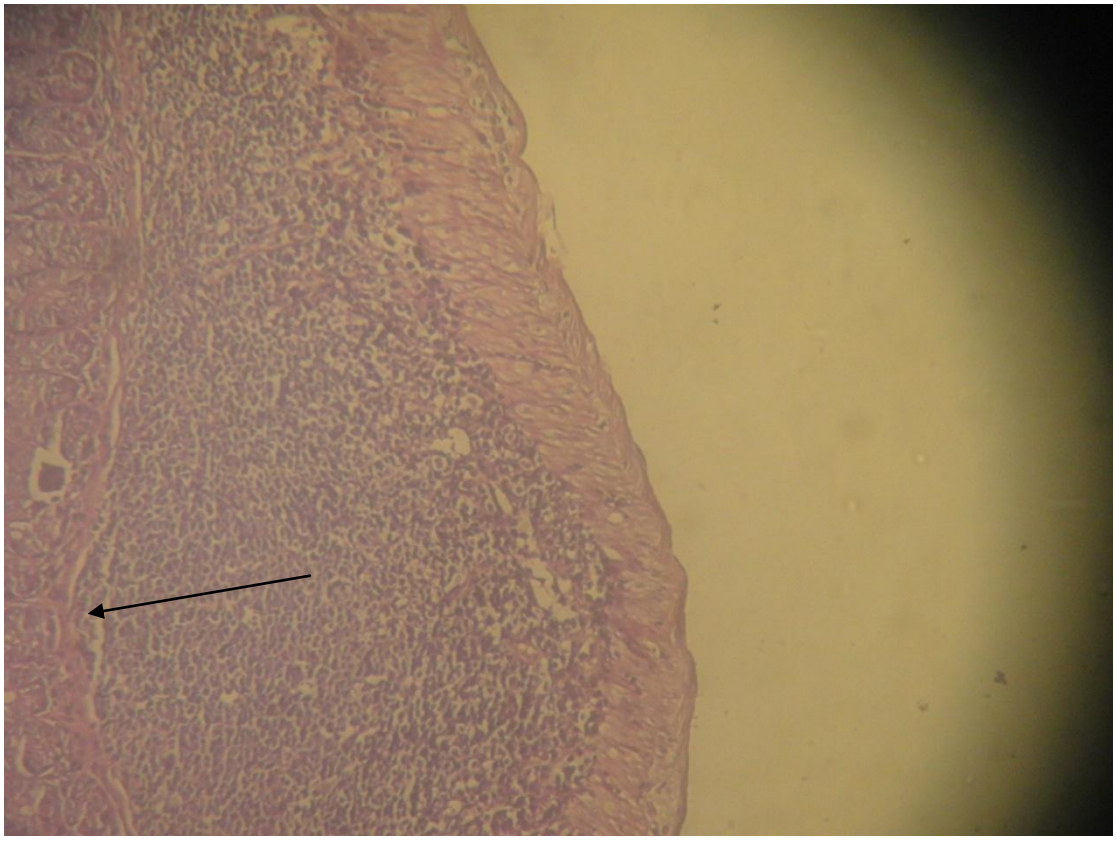

Figure 1: large lymph node in intestine mice tissue after expose to *Bifidobacteria* .H&E stain, 40x

DISCUSSION

In this study, a species of *Bifidobacterium* has been selected its to determine its effect on the *E. histolytica* in vivo. Selection of this bacteria was based on their use it in variety of fermented dairy products [27] as well as it was presence in the normal microflora of human [28] and the ability to resist stimulated small intestinal transit [29].

Bifidobacterium was successful as the meteronidazole in reducing *E. histolytica* cyst excretion over than the control group, *Bifidobacterium* may still have potential for therapeutic use against *cryptosporidium parvum* ; thus *Bifidobacterium* may produce the same or similar substances as *Lactobacillus acidophilus* and *L. veuteri* but in lesser and quantities [30]. The human guts colonized with a wide variety of microorganisms ,inducing species ,such as *Bifidobacterium* ,that have beneficial effects on human physiology and pathology .Among the most distinctive benefits of *bifidobacteria* were modulation of host defense response and protection against infections disease.

Bifidobacteria produce short-chain fatty acids (SCFAs) that have an antimicrobial effect by lowering the PH of bacterial cell growth [31].We propose that acetate produced by protective bifidobacteria improves intestine defense mediated by epithelial cells and there by protects the host against lethal infection [32].An important goal of therapy with bio therapeutic agents is to stop proliferation of the pathogen until such time that the normal microflora can be reestablished. The ability to "buy time " to reestablish colonization resistance is one probable important mechanism of successful therapy with bio therapeutic agents [33]. Generally accepted that *bifidobacteria* and *Lactobacilli* are important component of what might be termed the beneficial gut microbiota [34]. Our results show that that *Bifidobacterium* more efficient in treatment of *E. histolytica* than meteronidazole, also it has a protective effects. It has been know that the meteronidazole clinically more effective against amoeba in tissue than luminal amoeba [35]. Also it has been speculated that certain bacterial species of the gut may trigger the virulent potential

of the *Entamoeba* trophozoite while others may have no effect or may even cause a virulence [36].

Pérez *etal*. [37] showed that use 1,10,50 and 100 mg/ml of lyophilized conditioned with probiotics of *L. plantarum*, *L. casei* and *Bifidobacteria longum* considerate as probiotic showed significant difference on inhibition of growth of *E. histolytica*. The histopatholgical study shows that the lymph node in the intestinal tissue became obviously larger in mice inoculated with *bifidobacterium* , this result ,referred to the probiotics bacteria can induce immune modulation ,either through interaction with dendritic cells that can in furn modulate the differentiation of naïve T-cell ito Th1, Th2 or Treg lymphocytes leading to differentiate and induced to secret cytokines or through a humoral immune response via IgA producing cells and their secretory IgA [38]. The protective and treatment mechanism of the *Bifidobacterium* against *E. histolytica* with the nature and identity of the factor involved required in furthers study in the two levels of *in vitro* and *in vivo*.

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